REPORTS

CYP2E1 Genetic Polymorphisms and Risk of Nasopharyngeal Carcinoma in Taiwan

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Background: **Nasopharyngeal carcinoma occurs disproportionately among individuals of Chinese descent. The cytochrome P450 2E1 enzyme (CYP2E1) is known to activate nitrosamines and other carcinogens that are possibly involved in the development of this disease. Certain alleles of the CYP2E1 gene are thought to be more highly expressed than others, and their distribution varies between Asian and Caucasian populations. We conducted a case–control study to investigate whether such variations affect the risk of developing nasopharyngeal cancer.** ***Methods:*** **Three hundred sixty-four patients with nasopharyngeal carcinoma (96% of 378 eligible patients) and 320 control subjects (86% of 374 eligible subjects) were studied. A risk factor questionnaire was administered to participants to assess factors postulated to be linked to nasopharyngeal carcinoma. Peripheral blood was obtained from all subjects and DNA was purified from nucleated cells. A polymerase chain reaction-based restriction fragment length polymorphism assay that used the restriction enzymes *Rsa* I and *Dra* I was used to detect wild-type and variant forms of the CYP2E1 gene.** ***Results:*** **Individuals homozygous for an allele of the CYP2E1 gene that is detected by *Rsa* I digestion (c2 allele) were found to have an increased risk of nasopharyngeal carcinoma (relative risk [RR] = 2.6; 95% confidence interval [CI] = 1.2–5.7); this effect was limited to nonsmokers (RR = 9.3; 95% CI = 2.7–32) and was not affected by alcohol consumption.** ***Conclusions:*** **Our findings suggest that the CYP2E1 genotype is a determinant of nasopharyngeal carcinoma risk. [J Natl Cancer Inst 1997;89: 1207–12]**

Nasopharyngeal carcinoma is a rare tumor in most parts of the world, but occurs at relatively high rates in some geographic regions and among certain ethnic groups, with the highest incidence worldwide being reported from southeast Asia *(1)*. Numerous environmental factors have been associated with risk of developing nasopharyngeal carcinoma, including infection with the Epstein–Barr virus, occupational exposure to formaldehyde, cigarette smoking, and various dietary factors *(2)*. Host factors, including human leukocyte antigens and cytochrome P450 2E1 (CYP2E1), have also been postulated to be important in nasopharyngeal carcinoma development *(2,3)*.

CYP2E1 is an enzyme involved in the metabolic activation of various procarcinogens, including various low-molecular-weight nitrosamines, such as *N*-nitrosodimethylamine (NDMA) and the tobacco-specific nitrosamine *N*-nitrosonornicotine (NNN) *(4,5)*. The CYP2E1 gene is expressed and is inducible in hepatic tissue *(6)*. Studies *(7–11)* have also demonstrated the expression of CYP enzymes in the nasal epithelium of numerous animal species and humans.

The CYP2E1 gene is present in the population in various polymorphic forms. Polymorphisms detectable by *Dra* I and *Taq* I digestion are not thought to affect transcription or function of the enzyme coded for by the gene. In contrast, the variant detectable by *Rsa* I digestion (called the c2 variant) contains polymorphic base substitution sites in a region of the gene that is not transcribed but that appears to be involved in the transcriptional regulation of CYP2E1 expression. A study *(12)* has suggested that the variant form of the gene detectable by *Rsa* I digestion is expressed at higher levels than the wild-type form of the gene. Furthermore, individuals with the variant form of the gene have been shown in some studies to have higher hepatic CYP2E1 messenger RNA and protein levels and a greater ability to metabolize acetaminophen, a drug metabolized in part by CYP2E1 *(13–17)*. Interestingly, the c2 variant of the CYP2E1 gene has been shown to be present in approximately 20%–25% of Asians compared with fewer than 10% of Caucasians *(3,18–22)*. Furthermore, detection of the CYP2E1 c2 allele has been shown to correlate strongly with the presence of the rare allele detectable by *Dra* I digestion (C allele) *(3,22)*.

Recently, we reported an association between the presence of the *Rsa* I c2 and *Dra* I C alleles of the CYP2E1 gene and risk of nasopharyngeal carcinoma in a study of 50 nasopharyngeal carcinoma

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case subjects and 50 control subjects *(3)*. In this study, we have expanded our initial study to include 364 patients with nasopharyngeal carcinoma and 320 control subjects. This was done to 1) examine our initial findings with greater statistical power, 2) determine the independent effects of polymorphisms detectable by *Rsa* I and *Dra* I digestion, 3) explore the effect of CYP2E1 polymorphisms on risk associated with exposures to nitrosamine-containing cigarette smoke, and 4) consider the effect of ethanol, a known modulator of hepatic CYP2E1, on the CYP2E1/disease association.

Methods

Patients with histologically confirmed incident nasopharyngeal carcinoma were recruited over a 3½ year period from July 15, 1991, through December 31, 1994, at two large referral hospitals in Taipei, Taiwan: the National Taiwan University and MacKay Memorial Hospitals. Case subjects were eligible for study if they were less than 75 years of age, had had no prior diagnosis or treatment for nasopharyngeal carcinoma, and had resided in Taipei city or county for 6 months or longer. Three hundred seventy-eight eligible case subjects were identified.

For each eligible case subject, we attempted to match one population control subject. Three hundred seventy-four eligible control subjects were matched to eligible case patients. Matching was accomplished by use of listings available through the National Household Registration System. Each control subject was selected by visiting the registry office located in the district (if in Taipei city) or township (if in Taipei county) in which the case subject resided. Within these large geographic units, a random Li (communities of 1000–1500 households within each district/township) was selected. From within the selected Li, a random Lin (neighborhoods of 50–100 households) was selected. Individuals within the selected Lin were then enumerated and a control who matched the eligible nasopharyngeal carcinoma case subject on age (5-year groupings) and sex was randomly selected. Control subjects were required to have no history of nasopharyngeal carcinoma before identification for our study. Ineligible control subjects were replaced, but no replacement was attempted for eligible control subjects who refused participation.

All subjects were requested to respond to a personal interview that elicited detailed information on potential risk factors for nasopharyngeal carcinoma, including sociodemographic characteristics, cigarette smoking, alcohol consumption, dietary history, occupational history, and a family history of cancer. Case subjects were interviewed prior to treatment, at the time of their biopsies for confirmation of disease. In addition to the interview, subjects were asked to consent to the collection of approximately 30 mL blood. Three hundred sixty-nine case subjects (98%) and 320 control subjects (86%) gave verbal or written informed consent for both the interview and blood collection. This study was reviewed and approved by the Institutional Review Boards at the National Cancer Institute and the National Taiwan University.

For the 100 subjects reported on previously, DNA was isolated from peripheral blood mononuclear cells (PBMCs) obtained from whole blood and the polymerase chain reaction (PCR)-based restriction fragment length polymorphism assay was performed as described *(3)*. The remaining subjects were processed and tested as follows. Genomic DNA was isolated from PBMCs as previously described *(23)*. Amplification of the desired polymorphic sequences was carried out by use of PCR with the Perkin-Elmer PCR kit (The Perkin-Elmer Corp., Branchburg, NJ). The total volume of 50 μL consisted of 10 m*M* Tris (pH = 8.3), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 125 μ*M* deoxynucleoside triphosphate, 0.2 μ*M* primers, 2 U Taq polymerase, 0.4 μg TaqStart antibody (CLONTECH Laboratories, Inc., Palo Alto, CA), and 50–100 ng of template DNA. Primers were identical to those used previously *(3)*. Amplification was carried out for 30 cycles at 92 °C for 1 minute, 60 °C for 1 minute, and 72 °C for 2 minutes in a Perkin-Elmer Thermocycler. Twenty microliters of the 410-base-pair (bp) and 995-bp products were digested with 10 U *Rsa* I (410-bp product) or *Dra* I (995-bp product) (Boehringer Mannheim Corp., Indianapolis, IN) restriction enzyme overnight at 37 °C. The restricted product was run on a 10% precast Tris–Borate-EDTA nondenaturing polyacrylamide gel (Novex, San Diego, CA) at 100 V for 1.5 hours. Bands were visualized with an ultraviolet transilluminator after ethidium bromide staining.

Successful genotyping was obtained for 364 of the 369 case subjects and all 320 control subjects tested. A 6% masked, random sample (n = 42) of subjects were tested twice. The results were concordant for all masked duplicate sets. An additional group of 10 samples were tested twice, the first time using the method described in our previous publication *(3)* and the second time using refinements described herein to assure comparability. Results for all 10 samples were concordant.

Allele and genotype frequencies were computed. The Pearson correlation coefficient was used to determine the correlation between genotypic variables *(24)*. The relative risk (RR), as estimated by the odds ratio, was used to estimate the association between genotype or environmental exposures and disease *(25)*; 95% confidence intervals (CIs) were calculated to determine the statistical significance of findings *(25)*. Multivariate analyses were performed by use of logistic regression methods *(26)*. Stratified analyses were conducted to examine the joint effect of CYP2E1, smoking, and alcohol consumption. The significance of trends across multiple levels of an exposure was assessed by categorizing the variable of interest and treating the scored variable as a continuous variable in the regression model. Statistical significance of the observed interactions was determined by comparing the fit of the logistic model that included only the main effects with that of the model that included separate estimates of risk for CYP2E1 genotype for each level of the effect modifier (e.g., smoking). All *P* values resulted from two-sided statistical tests.

Results

A total of 364 case subjects and 320 control subjects are included in the analyses. The median age of the case subjects was 44.5 years (mean, 45.5 years; range, 15–74 years); 69.8% of the case subjects were male. Comparable statistics for control subjects were 45 years (mean, 46.0 years; range, 19–74 years) and 69.3% were male. Ethnically, 81.6% of the case subjects and 73.0% of the control subjects were of Fukienese origin; 13.2% of the case subjects and 11.0% of the control subjects were of Hakka, Cantonese, or Taiwanese (Aboriginal) origin; while the remaining 5.2% of the case subjects and 16.0% of the control subjects were of other Han origin ($P = .001$). Of the case subjects, 42.6% reported less than a junior high school education compared with 31.4% of the control subjects; conversely, 40.7% of the case subjects and 50.2% of the control subjects reported a high school education or higher (*P* for trend = .003). Other factors found to be associated with disease in our study or previous studies and considered as potential confounders included consumption of Guangdong (Cantonese) salted fish during childhood (RR = 1.4; 95% CI = 0.47–4.5), a family history of nasopharyngeal carcinoma (RR = 7.7; 95% CI = 2.3–26), and cigarette smoking (RR = 2.1; 95% CI = 1.2–3.7 for more than 30 years of use relative to nonsmokers).

The *Rsa* I c2 form of the CYP2E1 gene was observed among our control population with an allele frequency of 20.5% compared with 22.3% among case subjects ($P = .40$). Similarly, the *Dra* I C form of the CYP2E1 gene was observed with an allele frequency of 23.6% among control subjects and 25.4% among case subjects ($P = .64$). Both *Rsa* I and *Dra* I alleles were found to be in Hardy–Weinberg equilibrium. The distribution of the *Rsa* I and *Dra* I polymorphisms was observed to be similar in the different Chinese ethnic subgroups included in our study (Fukienese, Hakka, Cantonese, Aboriginal, and other Han).

Case and control subjects are compared with respect to CYP2E1 genotypic variants in Table 1. Twenty-seven case subjects (7.4%) and nine control subjects (2.8%) were homozygous for the *Rsa* I c2 form of the CYP2E1 gene; 30 case subjects (8.2%) and 14 control subjects (4.4%) were homozygous for the *Dra* I C form of the CYP2E1 gene. Those homozygous for the c2 variant were at a 2.6-fold increased risk of developing naso-

Table 1. Frequency distribution among case and control subjects and relative risk associated with genotypic variants of CYP2E1 detected by restriction enzyme digestion with *Rsa* I or *Dra* I

CYP2E1 genotype	Frequency		Relative risk (1)*	95% confidence interval	Relative risk (2)†	95% confidence interval
	Case subjects (n = 364)	Control subjects (n = 320)				
Rsa I						
c1-c1	229	198	1.0		1.0	
c1-c2	108	113	0.83	0.60–1.2	0.79	0.44–1.4
c2-c2	27	9	2.6	1.2–5.7	3.2	0.69–15
Dra I						
D-D	209	183	1.0		1.0	
D-C	125	123	0.89	0.65–1.2	1.1	0.61–1.9
C-C	30	14	1.9	0.98–3.7	0.81	0.20–3.3

*Controlled for age and sex.

†Controlled for age, sex, and the variables listed in the table (i.e., *Rsa* I adjusted for *Dra* I and vice versa).

pharyngeal carcinoma (95% CI = 1.2–5.7), while those homozygous for the C variant were at 1.9-fold increased risk of developing disease (95% CI = 0.98–3.7). Adjustment for education, ethnicity, a family history of nasopharyngeal carcinoma, consumption of Guangdong salted fish at a young age, or smoking did not materially affect our estimates of risk. Subjects heterozygous at the CYP2E1 locus (by either *Rsa* I or *Dra* I digestion) were not found to be at increased risk of disease relative to those homozygous for the more common allele (RR = 0.83 for *Rsa* I c1-c2 and 0.89 for *Dra* I D-C). Presence of the c2 and C variants of the CYP2E1 gene was highly correlated (σ_p = 0.85 among control subjects and 0.89 among case subjects). After adjustment for *Dra* I polymorphisms, individuals homozygous for the c2 variant of CYP2E1 were found to be at a 3.2-fold increased risk of disease (95% CI = 0.69–15). Conversely, adjustment of the *Dra* I effect for *Rsa* I polymorphisms resulted in an RR of 0.81 associated with homozygosity to the C form of CYP2E1 (95% CI = 0.20–3.3). Given these findings, further analyses focused on investigating the role of the c2 variant of the CYP2E1 gene on nasopharyngeal carcinoma risk.

Since cigarette smoking is an important source of nitrosamine exposure and alcohol consumption is known to modulate CYP2E1 activity, we examined the effect of these exogenous factors on disease risk and the joint effect of these factors and CYP2E1 polymorphisms on nasopharyngeal carcinoma risk. Smoking was positively associated with risk of nasopharyngeal carcinoma. Those reporting more than 30 years of smoking were at a 2.1-fold increased risk of disease compared with never smokers (95% CI = 1.2–3.7). Smokers (defined as subjects who reported smoking regularly for a period exceeding 6 months) who reported 30 or fewer years of smoking were at similar risk to never smokers (RR = 1.2; 95% CI = 0.80–1.8). No effect on disease risk was observed for alcohol consumption. Compared with nondrinkers, those who reported drinking two times per week or less and more than two times weekly had an RR of disease of 0.89 (95% CI = 0.56–1.4) and 0.92 (95% CI = 0.58–1.4), respectively. No association between alcohol consumption and disease was observed when the level of use was examined in finer strata.

We observed a strong and statistically significant modification of the CYP2E1 effect by smoking status (Table 2). Among never smokers, individuals homozygous for the CYP2E1 c2 variant were at a 9.3-fold increased risk of disease rela-

Table 2. Joint effect of CYP2E1 genotypic variants detected by *Rsa* I digestion and cigarette smoking/alcohol consumption on nasopharyngeal carcinoma risk

Smoking	c1-c1		c1-c2		c2-c2	
	No. of case/No. of control subjects*	Relative risk†	No. of case/No. of control subjects*	Relative risk†	No. of case/No. of control subjects*	Relative risk†
Nonsmokers	91/113	1.0	61/53	1.4	22/3	9.3‡
Smokers, y	138/84	2.3‡	47/59	1.1	5/6	1.1
≤15	32/27	1.6	14/13	1.3	2/1	2.4
16-30	62/37	2.2‡	17/31	0.70	2/3	0.84
>30	44/20	3.6‡	16/15	1.9	1/2	0.96
	Test for interaction: chi-squared = 23.44 (6 *df*); *P*<.001					
Nondrinkers	162/139	1.0	78/78	0.85	22/7	2.7‡
Drinkers	67/58	0.96	30/34	0.73	5/2	2.1
≤2 times/wk	34/28	0.98	14/17	0.68	2/1	1.7
>2 times/wk	33/30	0.94	16/17	0.78	3/1	2.4
	Test for interaction: chi-squared = 0.25 (4 *df*); *P*>.25					

*Two control subjects were excluded from analysis because of missing information on smoking history.

†Controlled for age and sex.

‡95% confidence interval excludes 1.0 (i.e., *P*<.05).

tive to nonsmokers who were homozygous for the CYP2E1 c1 allele (95% CI = 2.7–32). Conversely, among smokers, there was no evidence that being homozygous for the CYP2E1 c2 variant increased disease risk, relative to smokers who were homozygous for the wild-type gene, although this was based on only five case subjects and six control subjects who were both smokers and c2 homozygotes. When we restricted our analysis to never smokers who reported no passive smoking exposure in their household during childhood and adult life (n = 91), eight case subjects and no control subjects were observed to be homozygous for the CYP2E1 c2 variant. Furthermore, among these nonsmokers who reported no household passive smoke exposure, a significant increase in risk among individuals heterozygous at the CYP2E1 locus compared with individuals homozygous for the CYP2E1 c2 allele was observed (RR = 3.9; 95% CI = 1.4–11). When the RR of disease associated with cigarette smoking was investigated within strata of CYP2E1 genotype, a dose-related effect of years of cigarette smoking was observed only among individuals who were homozygous for the wild-type CYP2E1 gene. Among these subjects, risk increased from 1.6 for those reporting 15 or fewer years of cigarette smoking (95% CI = 0.82–2.9), to 2.2 for those reporting 16–30 years of use (95% CI = 1.3–3.8), to 3.6 for those who reported smoking for more than 30 years (95% CI = 1.8–6.9), relative to nonsmokers who were homozygous for the wild-type form of CYP2E1. Among subjects homozygous for the CYP2E1 c2 variant, a trend of decreasing risk with increasing years of smoking was observed, but this trend was not statistically significant (P = .13). Similarly, no significant trend of increasing or decreasing risk with increasing years of smoking was observed among individuals who were heterozygous at the CYP2E1 locus (P = .59). Parallel results were observed when intensity of cigarette smoking was examined, although the effect was not as striking, probably because intensity of cigarette smoking was not observed in our study population to be as strongly predictive of nasopharyngeal carcinoma risk as duration of use (data not shown). No effect modification was observed when the joint effect of CYP2E1 genotype and alcohol consumption was examined (P>.25; Table 2). Adjustment for cigarette smoking did not alter the alcohol/CYP2E1 findings. Our study size did not allow us to examine the joint effect on disease risk of consumption of salted fish or family history of nasopharyngeal carcinoma and CYP2E1 genotype.

We also examined the effect of CYP2E1 genotype separately for males (n = 476) and females (n = 208). A stronger association between homozygosity for CYP2E1 c2 and nasopharyngeal carcinoma was observed among females (RR = 17; 95% CI = 2.1–130) compared with males (RR = 1.2; 95% CI = 0.50–3.1), but this effect was largely explained by the fact that the majority of males reported a history of smoking (68.6%) while very few women reported having ever smoked (6.3%). When sex-specific analyses were performed separately for smokers and nonsmokers, the results were as follows. Excesses in risk of disease associated with being homozygous for the CYP2E1 c2 variant compared with being homozygous for the wild-type allele were observed for both sexes among nonsmokers; the risk estimate associated with homozygosity to the CYP2E1 variant was 16-fold among females (95% CI = 2.0–130) and sixfold among males (95% CI = 1.2–30). No effect of the CYP2E1 c2-c2 genotype was observed among male smokers (RR = 0.39; 95% CI = 0.11–1.4). Too few women reported a history of cigarette smoking (n = 13) to enable us to examine the effect of CYP2E1 genotype on risk of disease among female smokers.

Discussion

Results from this study confirm our preliminary report suggesting an association between CYP2E1 genetic polymorphisms and risk of nasopharyngeal carcinoma *(3)*. In the present study of 364 patients diagnosed with nasopharyngeal carcinoma and 320 control subjects, an RR of 2.6 was observed among individuals homozygous for the CYP2E1 c2 allele.

Since CYP2E1 is involved in the metabolic activation of numerous procarcinogens *(5)*, our finding of an association between CYP2E1 genetic polymorphisms and nasopharyngeal carcinoma also indirectly supports a role in nasopharyngeal carcinoma pathogenesis of procarcinogens known to be activated by CYP2E1. One such group of procarcinogens, nitrosamines, have previously been hypothesized to be linked to nasopharyngeal carcinoma *(2)*. Studies *(27–39)* have demonstrated an association between factors known to contain nitrosamines and nasopharyngeal carcinoma risk, an increased ability of individuals residing in high-risk areas for nasopharyngeal carcinoma to form nitrosamines endogenously, and the ability of nitrosamine-containing foods to induce nasal cavity tumors in animals.

In addition to nitrosamines, CYP2E1 is also involved in the activation of other procarcinogenic compounds *(5)*. The possibility that the CYP2E1/nasopharyngeal carcinoma association results from the ability of CYP2E1 to activate compounds other than nitrosamines should therefore not be overlooked. For example, CYP2E1 is involved in the metabolism of halogenated hydrocarbons, including organic solvents linked to the development of numerous tumors *(40)*. In fact, results from a recent study *(31)* suggested that exposure to solvents may be associated with nasopharyngeal carcinoma risk, although examination of the independent effect of solvents in that study was hampered by high correlations observed between solvent exposure and exposure to other occupational factors.

A provocative and unexpected finding of our study was the observation that the association between the CYP2E1 c2 allele and nasopharyngeal carcinoma risk was limited to nonsmokers and that, among nonsmokers who reported no household exposure to passive smoke, an effect was observed among individuals heterozygous at the CYP2E1 locus. Interpretation of this finding should be made with caution and requires confirmation. If confirmed, however, several explanations exist. One explanation for our finding is that tobacco products have inhibiting effects that counteract the effect of CYP2E1-induced activation. In fact, a recent study *(41)* has suggested that tobacco smoke constituents, including nicotine and cotinine, inhibit the activation of two nitrosamines present in cigarette smoke, NDMA and NNK, in a dose-dependent manner. It is also possible that the effect of polymorphisms in the CYP2E1 gene is more pronounced at low levels of exposure to ex-

ogenous factors. Finally, it is possible that CYP2E1 might not be important for the activation of tobacco-specific nitrosamines and that exposure to other procarcinogens, including other nitrosamines, that are activated primarily by CYP2E1 and that are associated with cigarette smoking might explain why the CYP2E1 effect is limited to nonsmokers in our study. In fact, laboratory data suggest that, in humans, liver metabolism of the tobacco-specific nitrosamine NNN is catalyzed mainly by CYP3A4 and CYP2A6, not CYP2E1, and epidemiologic data suggest that dietary factors are more important determinants of nasopharyngeal carcinoma risk than cigarette smoking *(2,42)*. As such, perhaps dietary nitrosamines, nitrosamine precursors, and/or other procarcinogens present in the diet might be important *(43–47)*. The low frequency with which the subjects in our study reported consumption of individual indicator food items in our questionnaire did not allow us to address this issue in the present report. However, efforts are under way to create composite food groups and nitrate, nitrite, and nitrosamine indices for subjects in our study using food-frequency questionnaire data.

In summary, our results demonstrate an association between CYP2E1 genetic polymorphism and nasopharyngeal carcinoma risk. The association was restricted to never smokers and was strongest among never smokers who also reported no passive smoking exposure in their household. Independent confirmation of this finding is required, and additional examination of the joint effect of CYP2E1 genotype and other non-tobacco-related exposures is needed before more conclusive interpretation of our results can be made.

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Correlation of Vascular Endothelial Growth Factor Expression and Microvessel Density in Cervical Intraepithelial Neoplasia

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Background: **Angiogenesis (the formation of new blood vessels) appears to be required for the growth of invasive tumors, but little information exists about its role in the development of preinvasive lesions. We examined microvessel density and expression of vascular endothelial growth factor in specimens of cervical intraepithelial neoplasia (CIN), a preinvasive lesion of the uterine cervix, to determine whether a connection could be established between these parameters of angiogenesis and the grade of dysplasia (i.e., tissue abnormality).** ***Methods:*** **Sections of biopsy specimens from 83 patients with grades I–III CIN were examined retrospectively. Microvessels were localized by use of a polyclonal antibody directed against factor VIII-related antigen; vascular endothelial growth factor was detected by means of a monoclonal antibody. Reported *P* values are two-sided.** ***Results:*** **Highest microvessel densities and highest expression of vascular endothelial growth factor were found in a narrow border region between CIN lesions and the underlying stroma. A significant correlation was observed between high vascular endothelial growth factor expression and high microvessel density (Kendall's τ = 0.27; 95% confidence interval [CI] = 0.03–0.50; *P* = .018). Mean microvessel density values ± standard deviations for CIN I, CIN II, and CIN III lesions were 19.4 ± 5.8, 21.9 ± 7.0, and 34.1 ± 14.8, respectively (Kendall's τ = 0.46; 95% CI = 0.30–0.61; *P*<.0001). Corresponding values for vascular endothelial growth factor expression were 8.3 ± 3.5, 8.4 ± 2.0, and 12.2 ± 3.6, respectively (Kendall's τ = 0.41; 95% CI = 0.20–0.60; *P*<.0001).** ***Conclusions:*** **Our results are consistent with the idea that progression of cervical dysplasia is dependent on angiogenesis. [J Natl Cancer Inst 1997;89:1212–7]**

The role of angiogenesis in tumor growth is well defined for a variety of solid malignant tumors *(1)*. Once a tumor has become established, a prevascular phase of uncertain length, during which the tumor is dormant, is followed by a phase of intense endothelial cell proliferation and subsequent tumor growth *(2)*. The angiogenic response is mediated by diffusible signals, so-called angiogenic factors, which can be secreted by malignant tumor cells *(2)*. Observations *in vivo* suggest that the inhibition of blood vessel formation results in a reduction in tumor size and a reduction in metastasis *(3–5)*.

The vascular endothelial growth factor (VEGF), which is also known as the vascular permeability factor (VPF), is a multifunctional cytokine that acts on endothelial cells as a highly specific mitogen *(6,7)*, binding to specific class III receptor tyrosine kinases (flt-1 and KDR) and opening calcium channels to increase intracellular calcium levels *(8)*. VEGF also stimulates angiogenesis by increasing vascular permeability *(7,9)*. Four VEGF isoforms (polypeptides containing 206, 189, 165, and 121 amino acids) have been isolated, but they apparently express identical biologic activities *(10)*.

Although extensive data are available on the role of angiogenesis in the development of a variety of invasive malignant tumors, little information exists concerning preinvasive lesions. Cervical intraepithelial neoplasia (CIN) is a preinvasive lesion of the uterine cervix that is charac-

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See "Notes" following "References."